FACTORS ASSOCIATED WITH BREAST CANCER AND ROLE OF NUTRITIONAL STATUS ON SERUM HIGH-MOLECULAR WEIGHT ADIPONECTIN

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FACTORS ASSOCIATED WITH BREAST CANCER AND ROLE OF NUTRITIONAL STATUS ON SERUM HIGH-MOLECULAR WEIGHT ADIPONECTIN

by

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMNS

Ab	Antibody
AdipoR1	Adiponectin receptor 1
AdipoR2	Adiponectin receptor 2
ADP	Adenosine 5′-diphosphate
ALDH2	Aldehyde dehydrogenase 2
AMP	Monophosphate-activated protein
apM1	Adipose most abundant gene transcript 1
ATP	Adenosine triphosphate
b	beta coefficient
BIA	Bioelectrical impedance analysis
BMR	Basal metabolic rate
BP	Blood pressure
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
cDNA	Complementary deoxyribonucleic acid
CI	Confidence interval
CL	Confidence limit
CRL	Central Research Lab
CRP	C-reactive protein
CV	coefficients of variability
CV_{wEI}	coefficient of variation of subjects' EI
CV_{wB}	coefficient of variation of repeated BMR measurement
CV_{tP}	coefficient of variation of total variation in physical activity

DHQ	Diet history questionnaire
DEXA	Dual energy x-ray absorptiometry
ECOG	Eastern Cooperative Oncology Group
EI	Energy intake
ELISA	Enzyme-linked immunosorbent assay
ER+	Estrogen receptor positive
FFQ	Food frequency questionnaire
HC	Hip circumference
HDL	High density lipo-protein
HER2	Human epidermal growth factor receptor 2
HMW	High-molecular weight
HRP	Horse radish peroxidase
Hs-CRP	High sensitivity C-reactive protein
HUSM	Hospital Universiti Sains Malaysia
H_2SO_4	Sulfuric acid
IARC	International Agency for Research on Cancer
LDL	Low density lipoprotein
LMW	Low- molecular weight
MAP	mitogen-activated protein
MMW	Medium-molecular weight
MNT	Medical nutrition therapy
MRI	Magnetic resonance imaging
MUFA	Monounsaturated fatty acid
NCR	National Cancer Registry
NHANES	National Health and Nutrition Examination Survey

NHMS	National Health and Morbidity Survey
OPD	O-phenylenediamine
OR	Odds ratio
PAL	Physical activity level
PR+	Progestrone receptor positive
PUFA	Polyunsaturated fatty acid
RNI	Recommended nutrient intake
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
TC	Total cholesterol
TCA	to come again
TEE	Total energy expenditure
TG	Triglyceride
UICC	Union for International Cancer Control
USM	Universiti Sains Malaysia
WC	Waist circumference

FAKTOR YANG BERKAITAN DENGAN KANSER PAYUDARA DAN PERANAN STATUS PEMAKANAN KE ATAS ADIPONEKTIN BERAT MOLEKUL TINGGI SERUM

ABSTRAK

Kanser payudara menjadi pembunuh utama wanita di Malaysia. Status pemakanan dan adiponektin adalah faktor risiko yang boleh diubah untuk kejadian kanser payudara yang boleh disasarkan dengan cekap. Tujuan kajian ini adalah untuk mengenal pasti faktor berkaitan kanser payudara dan perkaitan status pemakanan pada adiponektin berat molekul tinggi serum. Kajian ini adalah kajian kes-kontrol yang dijalankan dari Januari 2014 hingga Ogos 2015. Kajian ini dijalankan di Hospital Universiti Sains Malaysia (HUSM) dan Universiti Sains Malaysia (USM). Pesakit yang belum dirawat untuk kanser payudara (n=55) telah diletakkan ke dalam kumpulan kes, dan wanita sihat (n=58) yang merupakan kakitangan dari HUSM dan USM diletakkan ke dalam kumpulan kawalan. Data sosiodemografi dan reproduktif diperolehi melalui soal selidik yang piawai dan data pemakanan diperoleh dari soal selidik sejarah pemakanan yang disahkan. Penilaian antropometri (berat badan, ketinggian, lilitan pinggang, lilitan pinggul dan komposisi lemak badan) diukur dan sampel darah selepas puasa semalaman dianalisis untuk profil lipid, glukosa plasma, insulin, sensitiviti tinggi protein reaktif dan adiponektin berat molekul tinggi serum. Aktiviti fizikal diukur menggunakan akselerometer selama 2 hari bekerja dan 1 hari hujung minggu. Regresi logistik ringkas mendapati faktor-faktor yang dikaitkan dengan risiko kanser payudara adalah: sosiodemografi (umur, tahap pendidikan rendah, terdedah kepada asap rokok, pendapatan bulanan isi rumah yang rendah),

sejarah kesihatan (tekanan darah sistolik, tekanan darah diastolik, nadi, lebih dari 1 tahun penggunaan jamu) dan status pemakanan (ukur lilit pinggang, kolesterol HDL, trigliserida, glukosa darah dan pengambilan gula). Regresi logistik berganda dilakukan untuk menentukan faktor yang berkaitan dengan risiko kanser payudara. Faktor yang menyumbang kepada kanser payudara adalah perokok asap sekunder (OR = 10.00, [95% CI: 2.42, 41.30]), pendapatan bulanan isi rumah kurang daripada RM 2,300 (OR = 18.05, [95% CI: 2.56, 127.10]) dan tekanan darah sistolik (OR = 1.08, [95% CI: 1.04, 1.12]). Analisis regresi linear berganda menunjukkan terdapat hubungan negatif linear yang signifikan antara ukurlilit pinggang dan adiponektin berat molekul tinggi serum (β=-0.05; p=0.005) dalam kalangan kes-kes kanser payudara. Selain itu, kolesterol HDL secara positif dikaitkan dengan adiponektin berat molekul tinggi serum (β=1.83; p=0.010) dalam kalangan kes-kes kanser payudara. BMI dikaitkan secara negatif dengan adiponektin berat molekul tinggi serum (β=-0.02; p=0.001) dalam kalangan kumpulan kawalan sihat. Kajian ini menunjukkan faktor risiko yang boleh diubahsuai berperanan dalam berlakunya kanser payudara.

FACTORS ASSOCIATED WITH BREAST CANCER AND ROLE OF NUTRITIONAL STATUS ON SERUM HIGH-MOLECULAR WEIGHT ADIPONECTIN

ABSTRACT

Breast cancer is the leading killer of women in Malaysia. Nutritional status and adiponectin are modifiable risk factors for breast cancer occurrence that can be efficiently targeted. The purpose of this study was to determine the associated factor of breast cancer and relationship between nutritional status and high molecular weight (HMW) adiponectin. This was a case- control study, conducted from January 2014 until August 2015 at Hospital Universiti Sains Malaysia (HUSM) and Universiti Sains Malaysia (USM). Untreated breast cancer cases (n=55) were assigned as cases, and healthy controls (n=58) who were staff at HUSM and USM acted as controls. Sociodemographic and reproductive data were obtained with a standard questionnaire and dietary data was obtained from validated diet history questionnaire (DHQ). Anthropometric assessments (weight, height, hip, waist circumference (WC) and body fat composition) were measured and overnight fasting venous blood samples were analysed for lipid profiles, plasma glucose, insulin, high sensitivity C reactive protein (hs-CRP) and HMW adiponectin. Physical activity was measured using accelerometer for 2 weekdays and 1 weekend. Simple logistic regression found these factors associated with breast cancer risk: sociodemographic (age, lower education level, exposed to secondhand smoke, lower monthly household income), physical examination (systolic blood pressure, diastolic blood pressure, pulse, more than 1 year of traditional medicine) and nutritional status (WC, HDL cholesterol, TG, blood glucose and sugar intake). Multiple logistic regression was

performed to determine factors associated with breast cancer risk. The predictors of breast cancer development were exposed to secondary smoke (OR=10.00, [95% CI: 2.42, 41.30]), monthly household income of less than RM 2,300 (OR=18.05, [95% CI: 2.56, 127.10]) and systolic blood pressure (OR=1.08, [95% CI: 1.04, 1.12]). Multiple linear regression analysis revealed that there was a significant linear negative relationship between WC and HMW adiponectin (β=-0.05; p=0.005) among breast cancer cases. Besides, HDL cholesterol was positively associated with HMW adiponectin (β=1.83; p=0.010) among breast cancer cases. BMI was negatively associated with HMW adiponectin (β=-0.02; p=0.001) among healthy controls. This study indicated the potential role of modifiable risk factor in the occurrence of breast cancer.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Cancer is a name given to a pool of related diseases and can begin in almost any part of the human body (National Cancer Institute, 2015b). Cancer cells originated from abnormal cells. These cells can keep multiplying without control and invading other tissues. Some cancer cells can metastasize to distant sites. Cancer rates are increasing globally. Economically-developed countries are estimated to have higher new cases of cancer worldwide compared to economically developing countries (International Network for Cancer Treatment and Research, 2017).

Malaysia is a developing country with multi-ethnicity groups which has already reported to have 11% of principal cause of cancer mortality in women according to all medically certified deaths (Al-Naggar *et al.*, 2011). By the year of 2030, the global burden is expected by American Cancer Society to grow to 21.4 million new cancer cases and 13.2 million cancer deaths. In an effort to ease the global burden of cancer, World Health Organization (WHO) and International Agency for Research on Cancer (IARC) supports Union for International Cancer Control (UICC) to promote its ways through World Cancer Day on 4th February annually (Union for International Cancer Control, 2017).

The top 5 cancers affecting Malaysians are breast, colorectal, lung, cervical and nasopharyngeal cancer (National Cancer Society Malaysia, 2015). Breast cancer became the leading killer of women in Malaysia (25%), other than the Philippines (23%), Indonesia (22%), New Caledonia, Vanuatu (both 21%), Singapore (20%) and Samoa (13%) (Youlden *et al.*, 2014). It occurs in both men and women although male breast cancer is rare. Ductal carcinoma is the most common type of breast cancer which begins in the lining of the milk ducts. Other types of breast cancer is lobular carcinoma which initiates at the breast milk glands. However, invasive breast cancer has the tendency of spreading to surrounding normal tissue from its initial site that is located or confined to breast ducts or lobules (National Cancer Institute, 2015a).

A highly heterogeneous disease, breast cancer is a hormone-related disease in which the factors modifying the risk are different when breast cancer is diagnosed premenopausally or postmenopausally (Becaria Coquet *et al.*, 2016). However, it is impossible to identify the exact risk factors that cause the occurrence of breast cancer in certain women (Lacey Jr *et al.*, 2009). The survival of breast cancer can be improved through early detection which remains the cornerstone of breast cancer control (Anderson *et al.*, 2008). Nutritional status, the interplay of anthropometric assessment, dietary intake and physical activity are another important factor that may have impact on breast cancer risks. Weight gain has been frequently reported in breast cancer patients after diagnosis (Yaw *et al.*, 2011). Adherence to poor dietary intakes and physical inactivity are the important obesity-related risk factors on influencing breast cancer development. This is explained when the most common ways to combat obesity

are through nutrition and physical activity (Gershuni *et al.*, 2016). Lifestyle modification may perform a multi-targeted therapy to treat breast cancer incidence and recurrence as the individual component susceptive in fatality of breast cancer cannot be clearly evaluated (Chlebowski, 2013).

Adipokines are proposed to be key mediators in originating many obesity-associated malignancies (Katira and Tan, 2015). They are produced from stromal cells, macrophages and primarily from adipocytes which represent its mechanism through endocrine, paracrine and autocrine in the human body (Barone *et al.*, 2016). However, among these adipokines, adiponectin is known as multitalented player of innate immunity (Luo and Liu, 2016). Adiponectin plays a role in anti-diabetic effect, anti-inflammatory of atherosclerosis and lipid homeostasis (Liu *et al.*, 2013a). Previous epidemiologic studies led to the hypothesis that made adipokine become an inhibitor on the proliferation of various cell types by suppressing apoptosis, inhibiting inflammation and intensifying cell viability (Wang and Scherer, 2016). Adiponectin was ascertained two decades ago when there was a debate between adipose tissue and metabolic pathway. Though it has multitalented roles towards many organs, adiponectin played two main roles in liver and skeletal muscle by suppressing hepatic gluconeogenesis and promoting nutrient utilization (Wang and Scherer, 2016). Adiponectin may also have roles in maintenance of metabolic homeostasis and regulation of energy metabolism (Ruan and Liu, 2016). Mounting evidence revealed that adiponectin minimizes the risk of cancer.

1.2 Rationale of the study

Malaysia, one of the developing countries, is experiencing modernization and development towards the year of 2020. Given the population boom and aging in developing countries, the incidence of breast cancer is increasing sharply due to an increase in life expectancy, changes in reproductive factors, increased urbanization and adoption of western lifestyles (Shulman *et al.*, 2010). However, breast cancer problem was particularly prevalent as the top cancer in women both in the developed and the developing world (World Health Organisation, 2017). This increasing phenomenon suggests that new exploration is needed for many risks and underlying causes to breast cancer.

The contribution of research on various modifiable and non- modifiable risk factors established by conclusive research and confirmed by different sources help community to be aware at which risk level they are, in developing breast cancer. Nutritional status is suggested as one of the modifiable risk factors for breast cancer occurrence (Nomura *et al.*, 2016). The occurrence of breast cancer is stoppable if the modifiable risk factors can be overcome starting from adult life (Tamimi *et al.*, 2016). Factors that may affect the risk of cancer include dietary, physical activity, obesity, alcohol and environmental risk factor (National Cancer Institute, 2012). From this perspective, highlighting the nutritional status of women is therefore relevant to assist in predicting, declining and avoiding future breast cancer occurrence.

A study from Shahar (2010) had been conducted at Klang Valley (West Peninsular Malaysia) among newly-diagnosed breast cancer had similar study design. It is different from this study which has been conducted in Kelantan (East Peninsular Malaysia) that may have influence in lifestyle due to different culture and socioeconomic factors between west and east Malaysia. Furthermore, most studies on physical activity level amog breast cancer frequently focused on cancer survivors rather than untreated breast cancer patients. The findings on physical activity level using accelerometer were lacking among Malaysian untreated breast cancer women.

Circulating adiponectin level has been examined by many studies through meta-analysis as one of the associated risk factors of breast cancer (Ye *et al.*, 2014). However, it is currently an unknown mechanism on how adiponectin modulates breast cancer. Exploring the causal and functions of adiponectin may suggest a novel approach for breast cancer prevention. Yet, little is known about the adiponectin level among Malaysian untreated breast cancer women. Therefore, a study needs to be established to provide associated risk factors of breast cancer and impact of nutritional status on adiponectin among breast cancer cases and healthy controls.

1.3 Significance of the study

This study provides an updated data of health status between breast cancer cases and healthy controls. Discursive parameters related to the risk factors of breast cancer occurrence were analysed throughout the study including health status, dietary intake,

anthropometry measurement, body composition assessment and physical activity level. Besides, biochemical profiles of breast cancer and healthy controls were examined such as glucose, lipid profiles, C-reactive profiles, insulin and adiponectin. All these data add valuable information to the literature of breast cancer in Malaysia. Efforts in understanding and improving nutritional status may provide greater information on underlying mechanism that modulates breast cancer.

In future, the adiponectin biomarkers could be used by clinicians as a routine clinical practice to screen patients due to its therapeutic reactivation of anti-neoplastic signaling adiponectin (Surmacz, 2013). The study also can stimulate more research inspirations especially in the aspect of intervention study towards breast cancer survivors. The comparison data discovered through this case- control study is important to elucidate the potential causative factors of breast cancer in order to create guidelines of breast cancer prevention among Malaysian women. The exploration of potential risk of breast cancer would expand the knowledge associated to the mechanism modulated to breast cancer. The present findings would therefore provide conclusive evident and help health professionals generate approaches to lessen the health burdens of breast cancer among Malaysian women.

1.4 Objectives

1.4.1 General Objective

To determine the associated factor of breast cancer and role of nutritional status on high molecular weight (HMW) adiponectin.

1.4.2 Specific Objectives

i. To determine sociodemographic factors among breast cancer patients and healthy controls
ii. To determine physical examination and reproductive factors among breast cancer patients and healthy controls
iii. To determine nutritional status factors among breast cancer patients and healthy controls
iv. To determine the associated factors (sociodemographic, physical examination, reproductive and nutritional status) of breast cancer
v. To investigate the association between nutritional status and HMW adiponectin among breast cancer patients
vi. To investigate the association between nutritional status and HMW adiponectin among healthy controls

1.5 Research questions

i. Is there any association of the sociodemographic factors between breast cancer patients and healthy controls?

ii. Is there any association of the physical examination and reproductive factors between breast cancer patients and healthy controls?

iii. Is there any association of the nutritional status factors between breast cancer patients and healthy controls?

iv. Is there any factors associated with the breast cancer?

v. Is there any association between nutritional status and HMW adiponectin among breast cancer patients?

vi. Is there any association between nutritional status and HMW adiponectin among healthy controls?

1.6 Null hypotheses

i. There is no association of the sociodemographic factors between breast cancer patients and healthy controls

ii. There is no association of the physical examination and reproductive factors between breast cancer patients and healthy controls

iii. There is no association of the nutritional status factors between breast cancer patients and healthy controls

iv. There are no associated factors with the breast cancer in sociodemographic, physical examination, reproductive and nutritional status

v. There is no association between nutritional status and HMW adiponectin among breast cancer patients.

vi. There is no association between nutritional status and HMW adiponectin among healthy controls.

1.7 Conceptual framework

The conceptual framework of the study is presented in Figure 1.1. Two types of breast cancer risk consist of modifiable (sociodemography, reproductive and nutritional status) and non-modifiable risk factors (genetic make-up and reproductive). Modifiable risk factor is a type of risk that can be overcome through lifestyle changes while non-modifiable risk factor cannot be modified because it is not under one's control. An example for modifiable reproductive risk factor is breastfeeding practice and non-modifiable reproductive risk is age at menarche. Nutritional status interplay of anthropometry, biochemical marker, dietary intake and physical activity. Abnormal anthropometric measurement, unhealthy intake and physical inactivity as well as abnormal metabolic status contribute to higher risk of getting overweight and obesity. Moreover, anthropometric factors, dietary intakes also have relationship with adiponectin level. Obesity is one of the unhealthy lifestyle that may induce low adiponectin level (hypoadiponectinemia). Thus, low adiponectin level has an association with higher breast cancer risk.

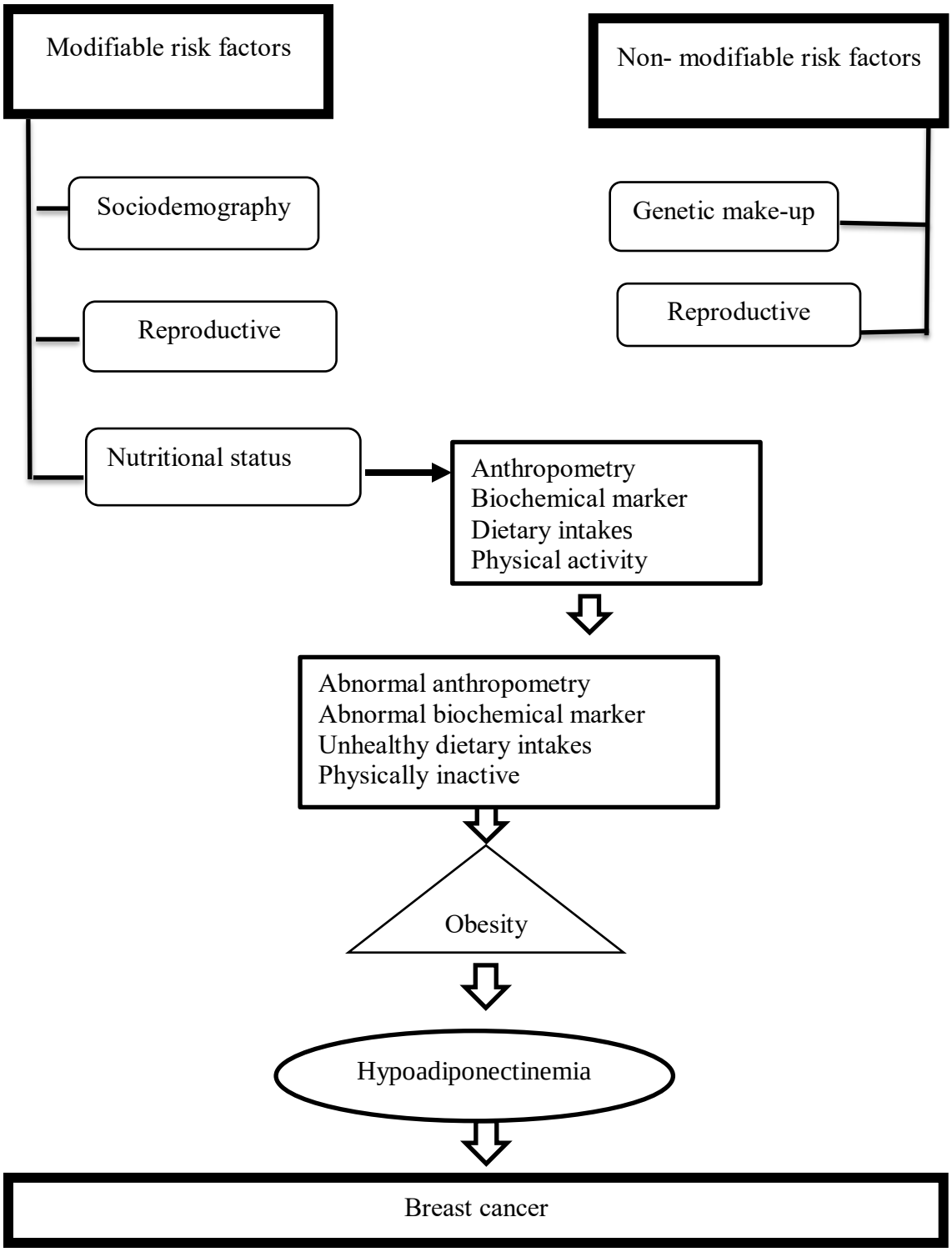

Figure 1.1 Conceptual framework of the study

(Adopted from Shahar *et al.*, 2010; James *et al.*, 2015; Tamimi *et al.*, 2016; current study)

CHAPTER 2

LITERATURE REVIEW

2.1 Breast cancer

Breast cancer is a type of disease in which tumor is formed from aggressive cells in the breast. Breast cancer origins may come from various parts of the breast. The most common are ductal and lobular cancer while other types of breast cancer are less common (American Cancer Society, 2017a). Up to 21 distinct histologic subtypes and at least 4 different molecular subtypes of breast cancer discovered (DeSantis *et al.*, 2016). Pathology report will confirm whether the breast cancer is a type of in situ or invasive. In situ breast cancers usually consists of ductal carcinoma in situ and lobular carcinoma in situ (confined within the ducts or lobules of the breast). Invasive breast cancer usually originated from the breast ducts or lobules which then infiltrate glandular walls or ducts to the nearest breast tissue (American Cancer Society, 2009).

Hormone receptor assay from pathology report provides result whether or not the breast cancer cells have receptors for the endocrine hormones. Estrogen receptor positive (ER+) and progesterone receptor positive (PR+) receive estrogen and progesterone signals to promote the breast cancer cells growth (Breast cancer.org, 2017). In addition, there is also an aggressive growing cancer due to high production of a protein known as human epidermal growth factor receptor 2 (HER2) (American Cancer Society, 2016a). Breast cancer patients may have the combination of these three

receptors called as triple positive breast cancer while triple negative breast cancer are not positive for estrogen, progesterone receptors, and HER2 (National Breast Cancer Foundation, 2016).

2.2 Prevalence of breast cancer

Breast cancer is one of the vital reproductive health problems among women (Forouzanfar *et al.*, 2011). Breast cancer is the most popular diagnosed cancer in women in which the cases are higher in less developed regions. Besides, it is the second most common cancer worldwide and being in the fifth ranks for the cause of death (Ferlay *et al.*, 2015). Women who underwent breast screening are prone to be diagnosed earlier with breast cancer than women who did not experience the screening. This approach may help one in eight women to be diagnosed earlier, spare the need for a mastectomy and avoid from breast cancer mortality (Screening, 2016).

Women at a younger age dying from breast cancer are rising in most countries especially in the developing world (Forouzanfar *et al.*, 2011) and it is a leading cause of death and disability among women in low- and middle-income countries (Porter, 2008). According to National Cancer Registry (NCR) 2011, breast cancer is the commonest cancer among females in Malaysia. Several Asian countries were reported to have rapid increase in breast cancer mortality rates particularly Malaysia (Youlden *et al.*, 2014). Despite a number of embattled therapies have been implemented in treating breast cancer, the breast cancer incidence and mortality rates remain on the rise, globally.

Breast cancer incidence were reported to increase by 3.1% yearly from 1980 to 2010 (Chong *et al.*, 2016).

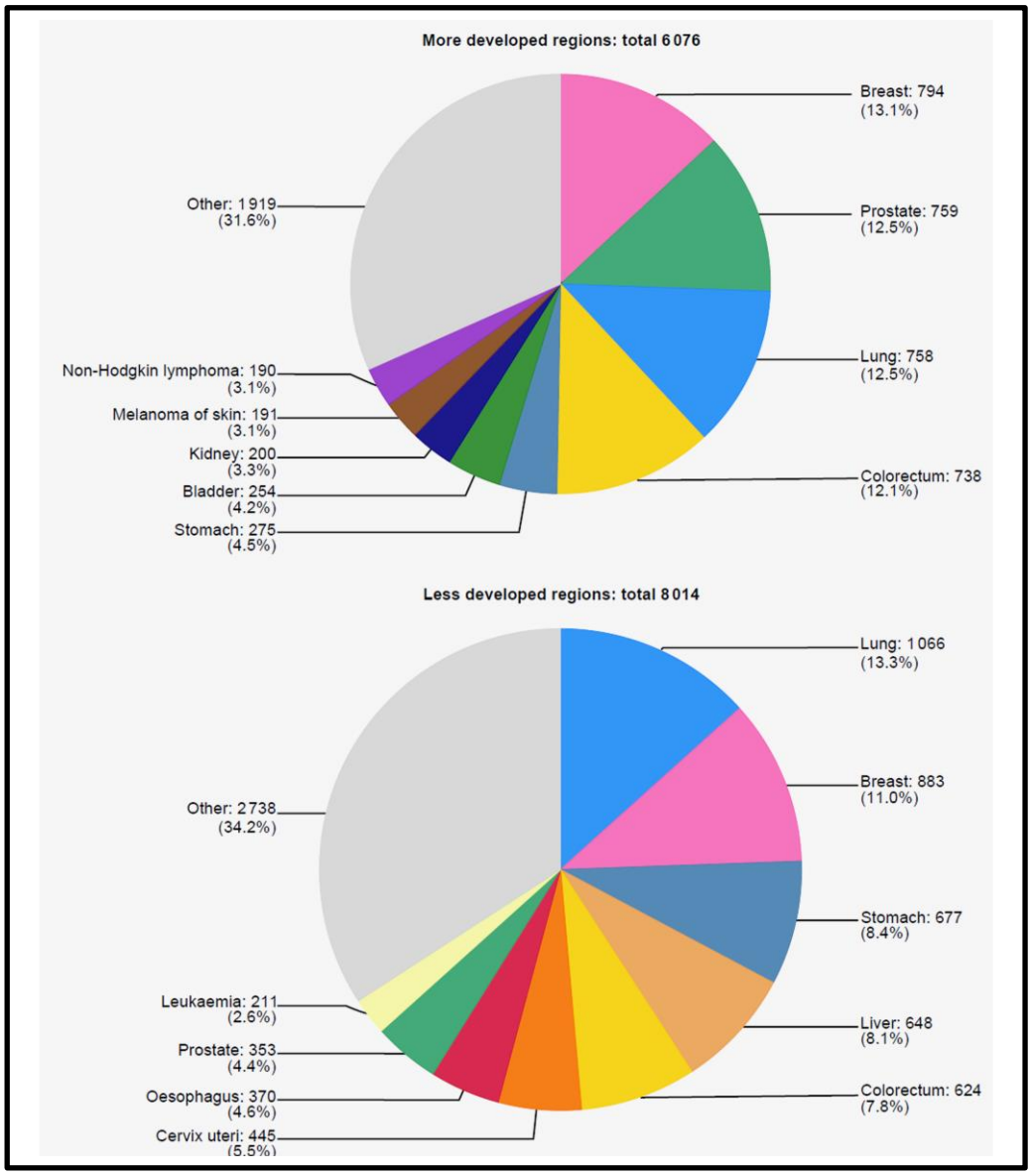

Figure 2.1 An estimated global numbers of new cases (thousands) with proportions for more developed and less developed regions

(Source: Ferlay et al., 2015)

2.3 Nutritional status

Nutritional status is an essential predictor of clinical outcome such as malnutrition. Impaired nutritional status was linked with inadequate food intake, unsustained hunger and metabolic inefficiency (Shakersain *et al.*, 2016). Several study modulated nutritional status as the assessment of anthropometry and body composition such as body mass index and body fat distribution (Bechard *et al.*, 2016). In contrast, nutritional status also has been defined in the scope of dietary intake by considering food intakes and utilisation of nutrients were affecting the consumer‘s health condition (Jeejeebhoy *et al.*, 1990).

Two main modifiable risk factors in prevention of cancer were body weight and dietary intake (American Cancer Society, 2016b). But then, the American Cancer Society provided new nutritional guidelines for the prevention of cancer by grouping the recommendations into categories of weight management, physical activity and dietary intake (Bail *et al.*, 2016). This recommendation may be used as nutritional education in promoting good health and optimum nutritional status among public. Moreover, nutritional status is one of the greatest concern towards the effort to increase patient’s quality of life. For example, Subjective Global Assessment (SGA) form has been utilized to screen patients with malnutrition in HUSM. This nutritional assessment tool may help beforehand in nutritional intervention to overcome disease complication (Sahran *et al.*, 2016).

2.4 Adipokines

Adipose tissue is a complex endocrine organ with convoluted role in homeostasis (Booth *et al.*, 2016). It can be epitomised up to 50% of the body weight (Peurichard *et al.*, 2017) and classified into white adipose tissue and brown adipose tissue (Hussein *et al.*, 2017).

White adipose tissue is one of the most abundant tissues in human in which abdominal subcutaneous adipose tissue and visceral adipose tissue are the main adipocyte depots of white adipose tissue (Bj *et al.*, 2011). On the other hand, additional white adipose tissue are distributed at disparate organs such as lung, heart, and kidney. White adipose tissue is designed from various type of cells including adipocytes, lymphocytes, endothelial cells, macrophages and fibroblasts (Kwon and Pessin, 2013). Its primary role is to store excess energy in the form of fat which then been utilised during energy deprivation and act as endocrine organ (Gesta and Kahn, 2017).

Brown adipose tissue exists abundantly in new born and hibernating mammals but almost absent in human adults (Coelho *et al.*, 2013). It is located in cervical-supraclavicular regions and contained higher number of mitochondria and capillaries. This enables brown adipose tissue to generate heat and dissipate energy (Ravussin and Galgani, 2011) through its mitochondria when the proton motive force across the inner membrane is unrestrained as heat rather than converted to ATP (Klingenspor *et al.*, 2017). Recent interest of studies on finding obesity solution suggested on the

conversion of fat accumulating white adipose tissue into energy-dissipating brown adipose tissue may be a potential harmless therapy to overcome obesity epidemic (Montanari *et al.*, 2017).

Subcutaneous or visceral adipose tissue (white adipose tissue) are responsible for the secretion of various factors known as adipokines, which can act locally or distally on other tissues (Kwon and Pessin, 2013). Adipokines are protein mediators secreted by adipose tissue (Leivo-Korpela *et al.*, 2011) and many grand new adipokines were discovered recently like adiponectin, leptin, and vaspin (Liu *et al.*, 2013). Adipose tissue has a role of multifunctional secretory organ which act to control energy homoeostasis through peripheral and central regulation as well as plays an important role in innate immunity (Piya *et al.*, 2013). Besides adipocyte, adipose tissue macrophages also become the main producers of adipokines (Capel *et al.*, 2009).

Adipokines can act centrally to regulate appetite and energy expenditure, and peripherally affect insulin sensitivity, oxidative capacity and lipid uptake (de Oliveira Leal and Mafra, 2013). At this point, adipokines are categorized as pro- and anti-inflammatory adipokines according to their effects on inflammatory responses in adipose tissues (Kwon and Pessin, 2013). Adipokines may have implicated through neuroendocrine, autonomic, or immune pathways in human body (Li *et al.*, 2013). Figure 2.1 shows adipose tissue plays endocrine role between organs. Adiponectin encourages insulin sensitivity in skeletal muscle and gives beneficial effects on cardiovascular and b-cell function of pancreas.

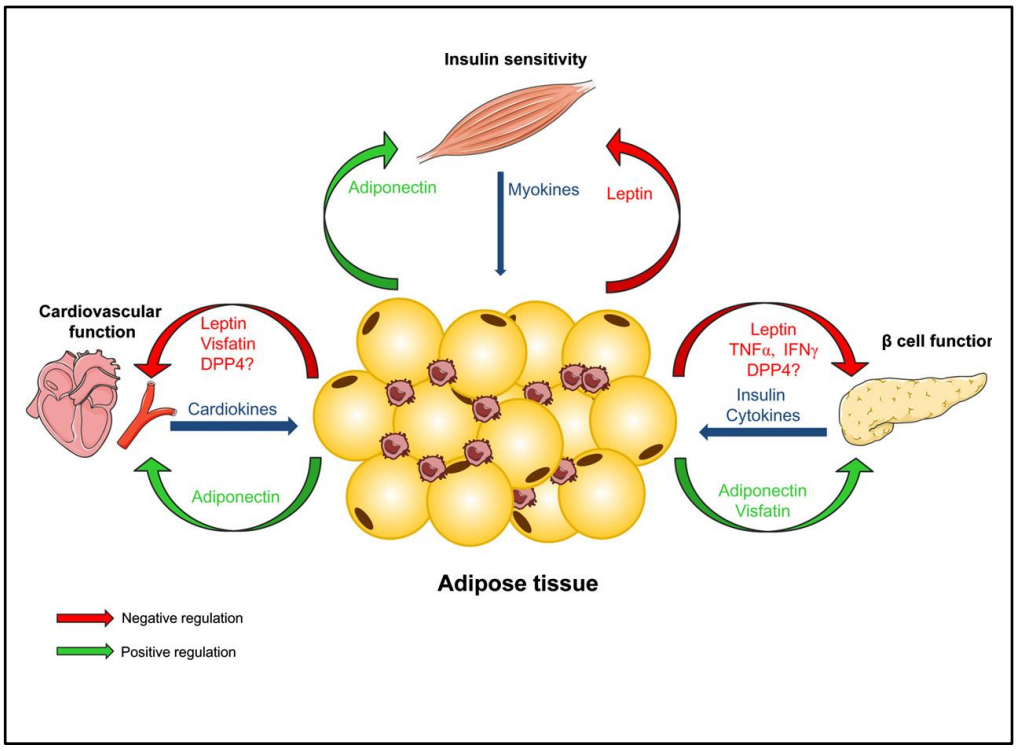

Figure 2.2 Positive regulation on multiple organs by adiponectin

(Source: Romacho *et al.*, 2014)

2.4.1 Adiponectin

Adiponectin, an adipocyte-derived adipokine was discovered over the past 20 years. Adiponectin (formerly named as adipocyte complement-related protein of 30 kDa, Acrp30) was revealed in 1995 from a subtractive complementary deoxyribonucleic acid (cDNA) library enriched in rodent adipocyte-specific genes (Scherer *et al.*, 1995). Then, in 1996, cDNA library from human adipose tissue was designed by Maeda *et al.*, (1996), also found adiponectin (named it adipose most abundant gene transcript 1 (apM1) and it is encoded by the ADIPOQ gene (Goto *et al.*, 2017). The human

adiponectin gene located on chromosome 3q26, an area associated with type 2 diabetes mellitus and metabolic syndrome susceptibility (Al Khaldi *et al.*, 2015).

2.4.1 (a) Structure and circulating levels

Nakano (1996) discovered circulating adiponectin when isolated from human plasma via gelatin-based affinity chromatography, high salt elution and protein sequencing. Full-length adiponectin comprises 244 amino acid, polypeptide, a collagen like domain at N-terminal secretory sequence, and a C-terminal globular domain with structural similarities to the complement factor C1q (Xu *et al.*, 2015). The adiponectin structure presented an asymmetric trimer of three identical monomers (Wang and Scherer, 2016). Post-translational modifications including hydroxylation and glycosylation were needed by full-length adiponectin to perform its role.

Adiponectin exists as multiple complexes with different molecular weights. The adiponectin molecules are circulated from adipocytes as trimers (~90 kDa; the basic unit); low molecular weight (LMW), hexamers (~180 kDa) and high molecular weight (HMW) isoforms (12–18-mer; >400 kDa) (Wang *et al.*, 2017). Figure 2.2 shows the structure of adiponectin consisted of three distinct complex forms, HMW (12–18mer), MMW (hexamer) and LMW (trimer). Matsuzawa (2010) reported that the average concentrations of adiponectin in human plasma are extremely high up to 5-10 μg/ml.

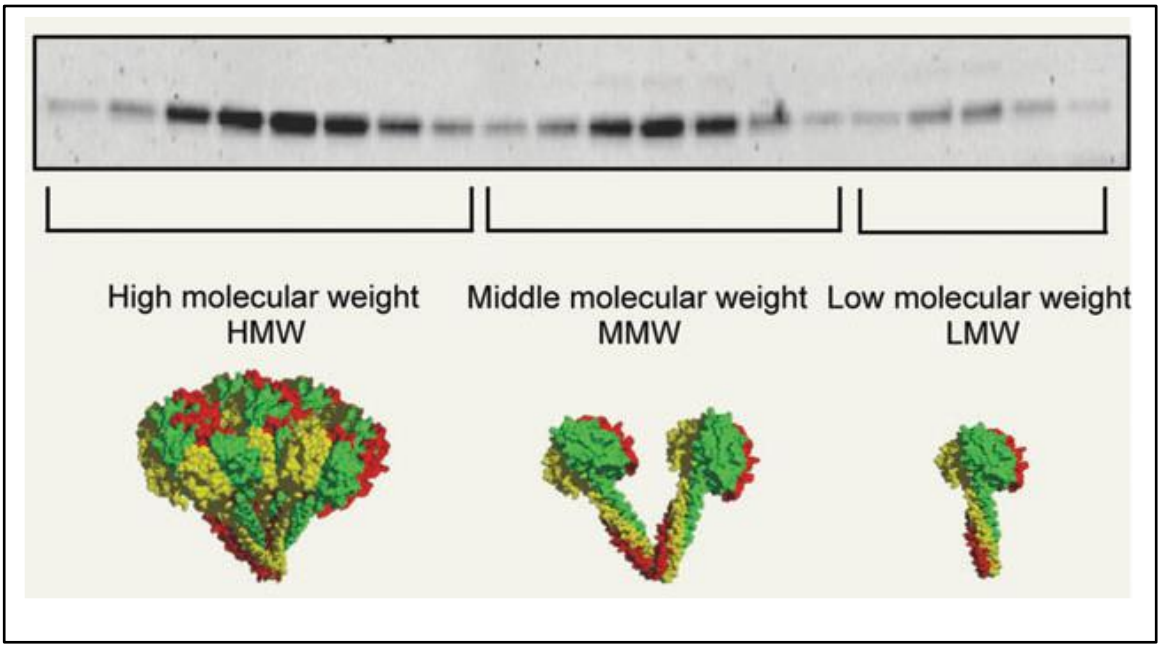

Figure 2.2 Structure of adiponectin

(Source: Wang and Scherer, 2016)

2.4.1 (b) Receptors

Adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2) are two transmembranous adiponectin receptor which contribute to biological effects of adiponectin such as inducing the activation of protein kinases, mainly monophosphate-activated protein (AMP) kinase and mitogen-activated protein (MAP) kinase (Yamauchi *et al.*, 2003). Besides that, AdipoR1 and AdipoR2 play roles in inhibition of breast cancer cells proliferation and induction of apoptosis (Dieudonne *et al.*, 2006). This has been strengthen with another study that resulted in AdipoR1 and AdipoR2 directly amend the growth of normal breast epithelial cells and breast cancer cells after determine the expression of AdipoR1 and AdipoR2 in the human breast epithelial cells

and breast cancer cells (Takahata *et al.*, 2007). Obesity will reduce adiponectin sensitivity due to reduction of adiponectin expression levels of AdipoR1 or AdipoR2, which finally leads to insulin resistance (Kadowaki and Yamauchi, 2005).

2.4.1 (c) Clinical features

Adiponectin found to be the most abundant transcript in visceral adipose tissue in the form of collagen-like protein that had bioactive substances. It plays multiple roles including anti-diabetic, anti-hypertensive, anti-inflammatory, anti-atherogenic properties and anti-oncogenic function (Matsuzawa, 2010). Silha *et al.* (2003) reported that decreased plasma adiponectin levels are found in obese compared with lean subjects. Adiponectin shown to demonstrate features of regulation lipid and glucose metabolism, growth of insulin sensitivity and protection against chronic inflammation (Liu and Liu, 2010). Thus, hypoadiponectinemia is associated with insulin resistance, hyperinsulinemia and the possibility of developing type 2 diabetes mellitus (Kaur, 2014).

The increasing percentage of obesity in the population has independent impact on cancer progression when previous study showed that fat tissue-derived adipokines, adiponectin has been involved in mammary carcinogenesis (VanSaun, 2013). It was found that adiponectin is the most abundant adipokine secreted by white adipose tissue (Xu *et al.*, 2016). It may limit tumorgenesis primarily by inhibiting cell metabolism and resisting detrimental leptin effects in breast cancer models (Surmacz, 2013). HMW

adiponectin is proposed to be the best predictor of metabolic parameters than total adiponectin. (Misra *et al.*, 2017) and is the only form that decreases after a glucose load, propounding that HMW adiponectin form is affected more rapidly than LMW or MMW form (Ozeki *et al.*, 2009).

Since adiponectin can be a suitable indicator of metabolic status, a study suggested developing adiponectin biomarkers in the clinic in similar ways as C-reactive protein. Clinically practice, high-sensitivity C-reactive protein (hs-CRP) was tested to predict cardiovascular risk which showed that both biomarkers had similar systemic readouts. Adiponectin biomarker may serve as a more stable metabolic dysfunction indicator as it is less prone to acute inflammator challenges than CRP (Kusminski and Scherer, 2009). Breast cancer patients also may benefit from adiponectin biomarkers due to its therapeutic reactivation of anti-neoplastic signaling adiponectin (Surmacz, 2013).

The association between adiponectin and obesity with breast cancer risk is well established discovery that culminate to speculation of adiponectin plays a role in breast cancer development. The first study in 2003 by Miyoshi *et al* reported the data of low serum adiponection levels are significantly associated with high risk of breast cancer. Furthermore, a case-control study conducted in Malaysia on roles of adiponectin in occurrence of breast cancer shows that low serum adiponectin was associated with breast cancer risk among subjects (Shahar *et al.*, 2010). However, another recent case-control study shows significantly higher of adiponectin level in newly diagnosed breast

cancer patients compared to controls, suggesting that this outcome may be due to the hypothesis that breast tissue can produce adiponectin (Al Awadhi *et al.*, 2012).

The first team conducted a case–control study determining the connection between adiponectin levels and breast cancer risk was Miyoshi *et al.* 2003. They acclaimed low level of serum adiponectin levels in breast cancer patients (7.57 µg/ml) compared to controls (8.83 µg/ml). According to meta-analysis, six studies were conducted in Asia while two from Europe. Results from these observational studies suggested that breast cancer cases had lower adiponectin level (95% CI:-0.618, -0.161; p value= 0.001) than controls (Ye *et al.*, 2014). According to systematic review and meta-analysis from 10 studies on dose-response analysis showed that an increase of 3 mg/ml of adiponectin corresponded to a 5% breast cancer risk reduction (Macis *et al.*, 2014).

Moreover, mean of HMW adiponectin level among Japanese women were reported significantly lower in breast cancer cases compared to controls in regard to menopausal status. In premenopausal women, the mean HMW adiponectin concentration was 4.2 µg/ml among cases and 7.6 µg/ml among controls while in postmenopausal women, it was 5.2 µg/ml and 9.2 µg/ml among cases and controls (Minatoya *et al.*, 2014). Assiri *et al.* 2015, reported that adiponectin was a risk factor for breast cancer in both premenopausal and postmenopausal women. More than that, the risk of breast cancer among postmenopausal women were increased in those with obese problem (Chen *et al.*, 2006).

2.5 Predictor factors associated with the occurrence of breast cancer

Research into the causes of breast cancer has progressed to the point of risk markers to distinguish between women at different levels of risk which resulting into modifiable and non-modifiable risk factors. Non- modifiable risk factors of breast cancer consist of age, family history, age at first full-term pregnancy, early menarche, late menopause, and breast density. Other factors associated with increased breast cancer risk are modifiable such as physical inactivity, alcohol consumption and postmenopausal obesity (American Cancer Society, 2017b).

Based on the breast cancer health disparities study, both modifiable and non-modifiable risk factors are inter-related. Dietary factors had relationship with angiogenesis-related genes which led to breast cancer carcinogenesis (Slattery *et al.*, 2014). Moreover, modifiable factors also had effect on specific breast cancer subtype. Poor prognostic triple negative tumors may be prevented through balanced healthy weight while extended breastfeeding period and higher parity may minimize luminal breast cancer (Turkoz *et al.*, 2013).

Risk factors of breast cancer occurrence are divided into 3 major categories which are genetic, reproductive and environmental factors (Ban and Godellas, 2014). This study specifically divides risk factors into modifiable and non-modifiable factor.

Focus was given to genetic risk (non-modifiable), nutritional risk (modifiable) and reproductive (modifiable and non-modifiable).

2.5.1 Genetic risk of breast cancer

The risk of breast cancer at different levels may be identified through data bank of multiple common susceptibility alleles for cancer of breast (Mavaddat *et al.*, 2015). The most famous susceptibility genes of breast cancer being studied for breast cancer are BRCA1 and BRCA2 (BReast CAncer). Both BRCA1 and BRCA2 are initially been transcripted to prevent from cancer occurrence but any mutation occurred in any BRCA1 and BRCA2 or both may lead those carrier to breast cancer exposure (Centers for Disease Control and Prevention, 2014). About 45 to 80% lifetime risk among women carrying BRCA1 or BRCA2 gene mutation risky to get breast cancer (Geyer *et al.*, 2017). Most of the BRCA1 allied with triple negative breast cancer while BRCA2 cancers associated with ER+ (Macedo *et al.*, 2016).

A genetic testing study of 60,000 female patients with breast cancer was conducted via hereditary cancer gene panels to determine germline cancer predisposing mutations. As a result, 9% of women were detected with mutations in which moderate risk (RR>2.0) of breast cancer association with *ATM, RAD51D, NF1,* and *MSH6* and high risk (RR>5.0) association with genes *BRCA1, BRCA2, PALB2 (Couch et al., 2017).* This large genetic testing study contributed in providing functional data in hereditary cancer gene panels and accumulated risk estimates for many predisposition